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Research Article

Comparison of Serum Oxidative Stress Markers of Malondialdehyde and Total Antioxidant Capacity Between Women with Infertility Due to Polycystic Ovary Syndrome and Tubal Factor

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Abstract

Objective: To compare mean serum level of malondialdehyde (MDA) and Total Antioxidant Capacity (TAC) in women with anovulatory infertility due to Polycystic Ovary Syndrome (PCOS) and those with tubal factor infertility.

Methods: It was nested case-control study of infertile women with confirmed anovulatory infertility due to PCOS and age-matched women with confirmed tubal factor infertility (controls) over a 6-month period. Serum MDA and TAC levels were estimated and compared between the two groups. A correlation test was done between Body Mass Index (BMI) of the participants and the serum levels of these oxidative stress markers. The data was analyzed using statistical package for social science version 24.0.

Results: A total of 111 subjects (63 cases and 48 controls) were finally used for analysis. The mean serum level of MDA is significantly higher in the case group than the control group (3.01±0.99 vs. 2.36±0.79nmol/mL; P < 0.001); however, the mean serum level of TAC was significantly lower in the case group than the control (692.19±133.89µmol/L vs. 932.94±201.28µmol/L; P < 0.001). There was a weak correlation between serum MDA and BMI (r = 0.189, p=0.138), and between TAC and BMI (r =0.108, p=0.399).

Conclusion: Women with anovulatory infertility due to PCOS have significantly higher serum levels of MDA and lower serum levels of TAC than women with tubal factor infertility. This supports a possible role of oxidative stress markers in the etiology and pathogenesis of PCOS infertility. Antioxidant supplementation may be beneficial in the control and management of anovulatory infertility due to PCOS.

Key words: Anovulatory infertility; malondialdehyde; oxidative stress; polycystic ovary syndrome; total antioxidant capacity

List of Abbreviations: AOPPs: Advanced Oxidation Protein Products; BMI: Body Mass Index; cGMP: Cyclic Guanosine Monophosphate; COS: Controlled Ovulation Stimulation; DNA: Deoxyribonucleic Acid; dROMs: Reactive Oxygen Metabolites; FRAP: Ferric Reducing Ability of Plasma; GCs: Granulosa Cells; GPx: Glutathione Peroxide; ICSI: Intracytoplasmic Sperm Injection ; IUGR: Intrauterine Growth Restriction; MDA: Malondialdehyde; NAUTH: Nnamdi Azikiwe University Teaching Hospital; OS: Oxidative Stress; PCOS: Polycystic Ovary Syndrome; PCR: Polymerase Chain Reaction; ROS: Reactive Oxygen Species; SOD: Superoxide Dismutase; TAC: Total Antioxidant Capacity; TBA: Thiobarbituric Acid

Introduction

Polycystic Ovarian Syndrome (PCOS) is a heterogeneous and heritable disorder that affects women throughout their entire lifetime. It predisposes to diabetes mellitus, cardiovascular problems, and endometrial cancer among other complications. PCOS accounts for about 75% of ovulatory factor infertility in women of childbearing age [1-3]. Globally, it has prevalence of 5-10% [2]; with some racial/ethnic variations. The prevalence rate of PCOS in Nigerian population varies from 3.2% to 18.1% [4,5]. The diagnosis is usually made using the Rotterdam's criteria [2]; which utilizes 2 out of the 3 of these criteria: oligo/anovulation, clinical and/or biochemical evidence of hyperandrogenism, and polycystic ovary morphology. For a reliable diagnosis of PCOS, several other conditions that could cause similar symptoms of menstrual dysfunction must be ruled out. The treatment of PCOS is individualized per the patient's health needs and priorities [2,5]. Oxidative Stress (OS) has been implicated in its etiopathogenesis [6]. These oxidative stress markers include Malondialdehyde (MDA), Superoxide Dismutase (SOD), and Glutathione Peroxidase (GPx) [7,8]. It occurs because of an imbalance between the generation of free radicals and the body's ability to neutralize them by the antioxidants. It has been implicated in many reproductive pathologic processes [9]. Malondialdehyde (MDA), which is a marker of oxidative stress, is a stable product formed by the peroxidation of polyunsaturated fatty acids of cell membrane [10]. The degree of antioxidant defense present in a biological system is referred to as Total Antioxidant Capacity (TAC). It has been shown that increase in MDA and decrease in TAC indicates an increase in oxidative stress [11]. Several studies have demonstrated the theory of the etiological association between Reactive Oxygen Species (ROS) and anovulation in PCOS patients with infertility [12-17]. When ovarian follicles experience oxidative stress, it can lead to direct damage to oocytes leading to impaired fertilization [12]. Despite this implication of oxidative stress in female infertility, there is dearth of comparative data on OS in women infertility of different aetiologies. Although a recent nested case-control study by Enechukwu et al. [13] determined the activities of oxidative stress markers and lipid profiles of patients with PCOS, the control group consisted of healthy women without infertility. Thus, the findings of Enechukwu et al study were apparent from the onset as better control would have been patients who had infertility apart from PCOS [13]. To expand the current literature on the exclusive contribution of oxidative stress to an etiopathogenesis of anovulatory infertility due to PCOS, the present nested case-control study was conducted to evaluate the serum oxidative stress markers and antioxidant capacity among PCOS patients with anovulatory infertility in comparison with infertile women with tubal factor infertility without PCOS.

Materials and Methods

Study design

A nested case-control study.

Study setting

Nnamdi Azikiwe University Teaching Hospital, Nnewi, South-east Nigeria.

Study population

The study involved consented patients with infertility (anovulation due to PCOS as the test arm and tubal factor infertility as the control group).

Study duration

The study lasted for 6 months.

Diagnostic criteria

The diagnosis of PCOS was made according to the Rotterdam criteria, as defined by the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine consensus declaration of 2003 [2,13]. The diagnostic criteria employed at least 2 out of the following 3 features: 1) Oligo- and/or anovulation; 2) Clinical and/ or biochemical evidence of hyperandrogenism; and 3) Transvaginal ultrasound scan morphology of PCO with 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume >10 mL [2,13]. All the patients did hormonal profile to aid the diagnosis. Tubal factor infertility was diagnosed by hysterosalpingography and/or laparoscopy and dye test. The exclusion criteria were women diagnosed with chronic hypertension, cardiovascular diseases, diabetes mellitus, endometriosis and thyroid dysfunction, and women diagnosed with PCOS who had been on hormonal therapy, lipid-lowering or insulin-sensitizing drugs over the previous 3 months were also excluded.

Sample size

We estimated that a sample size of 84 with a 1:1 case to control ratio (42 cases and 42 controls) would allow us to accept a two-tailed alpha error of 0.05 with 80% power using mean of 347.5

nmol/l and standard deviation of 22.8nmol/l for women with PCOS infertility and decrease of 4% in the MDA levels for controls as reported in a previous study by Turan et al. [14]. However, we recruited a total of 111 participants (63 and 48 participants in the case and control groups respectively to account for possible loss of sample and withdrawal of consent. Within the study period, 360 patients presented to fertility clinic of which 70 meet the criteria for PCOS and 190 for tubal factor infertility. After thorough evaluation and exclusion, 63 were recruited for assessment of MDA and TAC on the PCOS arm while 48 participants were recruited for the tubal factor arm. See Figure 1 for details.

Outcome measure

The mean serum level of MDA and total antioxidant capacity were the primary outcome measures.

Procedure and Data Collection

The socio-demographic variables were extracted using a proforma. The height and the weight of the participants were obtained; and their body mass index calculated using weight (kg) / height (m2). The body weight of each subject in kilograms (kg) was determined using a clinical weighing scale, while the height in meters (m) was measured using a stadiometer. Body Mass Index (BMI) was calculated by dividing the weight by the square of the height (kg/m2). Blood was collected in a plain sterile vacutainer tubes and serum extracted after centrifugation. The sample was allowed to clot at room temperature for 15minutes. The clot was removed by centrifugation at 1,500xg for 10minutes by the researcher. The resulting supernatant (serum) was immediately transferred into a clean tube and then stored at -20oC until analysis was done.

Determination of Malondialdehyde (MDA) level.

The determination of MDA level was carried out using the colourimetric method of Gutteridge and Wilkins (1982). This method was used because it is readily available and is also acceptable. Other more sophisticated methods of analysis were not available in our hospital.

a) Principle: Malondialdehyde (MDA) is a product of lipid peroxidation. When heated with 2-thiobarbituric acid (TBA) under alkaline condition, MDA forms a pink-colored product, which has maximum absorption at 532 nm. The intensity of colour generated is directly proportional to the concentration of MDA in the sample. To 0.1 ml of sample in test tube, 1 ml of 1% Thiobarbituric Acid (TBA) dissolved in alkaline medium (0.05 M sodium hydroxide) was added. The mixture was thoroughly mixed, and 1 ml of glacial acetic acid will be added to the mixture, thoroughly shaken, and incubated in boiling water (100 °C) for 15 minutes. It will be allowed to cool, and the turbidity was removed by centrifugation at 3000 rpm (revolution per minute) for 10 minutes. Thereafter, the supernatant was read at 532 nm. The same volume of TBA and glacial acetic acid was added to the blank, but 0.1 ml of distilled water was used instead of plasma.

b) Calculation:

The level of MDA in the serum was expressed as nmol/ml using the molar extinction coefficient for MDA (1.56x105M-1cm-1).

MDA (nmol/ml) = (OD X 1000000)/ E532

Where:

E532 = Molar extinction coefficient for MDA (1.56x105 M-1cm-1)

1000000 = conversion of mMol to nMol

Estimation of Total Antioxidant Capacity.

a) Principle of the Test: Total antioxidant activity was estimated using Ferric Reducing Ability of Plasma (FRAP) method by Benzie and Strain, 1996. At low pH, antioxidant power causes the reduction of ferric tripyridyl triazine (Fe III TPTZ) complex to ferrous form (which has an intense blue colour) that can be monitored by measuring the change in absorption at 593nm. FRAP values were obtained by comparing the absorbance change at 593 nm in mixture (test), with those containing ferrous ion in known concentration (Standard).

b) Procedure: A working reagent comprising acetate buffer (pH 3.6), ferric chloride and tripyridyl triazine in the ratio of 10:1:1 respectively was constituted. To 60 µl of sample or standard in a clean test tube, 1.8 ml of working reagent was added, thoroughly mixed, and incubated at 37 °C for 10 minutes. The resulting blue colored solution that was developed was read at 593 nm. The standard was also be treated the same way except that 60 µl of distilled water was used instead of plasma. The standard solution contains 1000 µmol/l of ferrous sulphate (Table 1).

c) Calculation:

$$\text{Total Antioxidant Capacity (µmol/l)} = \frac{\text{OD TEST}}{\text{OD STD}} \text{ X standard concentration (1000).}$$

d) Statistical analysis

The data was analyzed using SPSS (Statistical Package for Social Sciences) version 24. Paired t-Test was used to assess the significance of difference of the mean values of different parameters in the case and control groups. A correlation test was done between BMI of the participants and the serum levels of these oxidative stress markers.

e) Ethical approval

The study was approved by Nnamdi Azikiwe University Teaching Hospital Ethics Committee (approval number: NAUTH/CS/66/VOL.10/225/2017/139; approval date: 22nd January 2018).

Table 1: Distribution of socio-demographic parameters of test and control groups of the participants.

Age Range	Case (%)	Control (%)	X2-value	P-value
<21-25 years	13(11.22%)	11(10.60%)		
26-30years	21(19.72%)	15(13.51%)		
31-35years	17(11.71%)	13(15.32%)	24.696	0.08
36-40years	9(7.31%)	8(8.11%)		
41years & above	3(1.80%)	1(2.70%)		
	63(49.76%)	48(50.24%)		
BMI				
Normal weight	21(18.92%)	19(17.12%)		
Over weight	36(32.43%)	29(26.13%)	46.108	0.001
Obese	6(5.41%)	0(0.00%)		
	63(56.76%)	48(43.24%)		
Occupation				
C/S	20(18.02%)	12(10.81%)		
Housewife	2(1.80%)	6(5.41%)		
Trader	21(18.92%)	29(26.13%)	60.407	0.001
Student	15(13.51%)	1(0.90%)		
Others	5(4.50%)	0(0.00%)		
	63(56.76%)	48(43.24%)		
Education				
Primary Education	1(0.91%)	4(3.64%)		
SSCE Education	35(31.82%)	29(26.36%)	47.036	0.001
Tertiary Education	27(23.64%)	15(13.64%)		

Key: BMI: Body Mass index; C/S: Civil Servants; SSCE: Senior Secondary Certificate Examination.

Results

During the study period, from October 1, 2018, to March31, 2019, a total of three hundred and sixty (360) infertile women presented to the fertility clinic of the hospital; of which 63 PCOS patients were selected for the study, and 48 patients of tubal factor infertility as control. See Figure 1. The mean age of the case group was (28.84±5.76 years) while that of the control group was (31.66±5.26 years). Table 2: Paired T-test analysis comparing the mean serum levels of malondialdehyde and total antioxidant capacity of PCOS patients and controls. There was a significant difference in the mean levels of MDA and TAC between cases and control (P=0.001). Figure 2 shows the scatter diagram shows the correlation between the serum malondialdehyde (nmol/mL) (Y-axis) and body mass index (kg/m2) (X-axis). The result from the plot showed that there is very weak correlation between serum malondialdehyde and body mass index (r = 0.189, p >0.138). Figure 3 shows a scatter diagram revealing the correlation between the Serum Total Antioxidant Capacity (µmol/L) (Y-axis) and Body Mass Index (Kg/m2) (X-axis). The result from the plot showed a very weak correlation between the Serum Total Antioxidant Capacity (µmol/L) and Body Mass Index (r = 0.108, p > 0.399) (Table 3).

Table 2: T-test analysis comparing the mean serum levels of malondialdehyde and total antioxidant capacity of PCOS patients and controls.

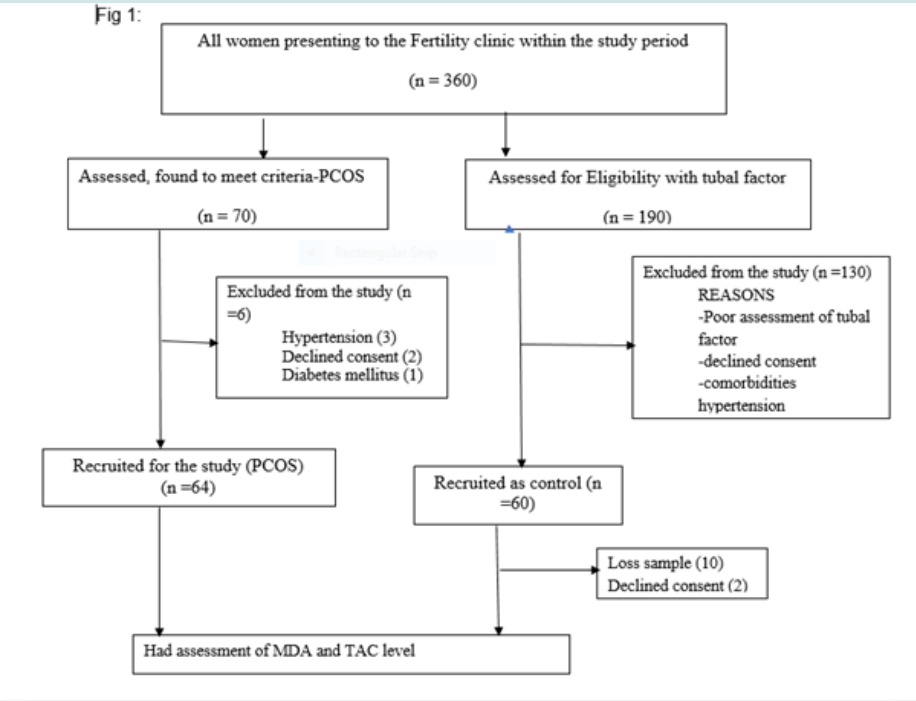
Parameters	Group	Mean±Std	P-value	t-value
	Case	3.01±0.99	0.001	3.82
MDA (nmol/mL)	Control	2.36±0.79		
TAC (µmol/L)	Case	692.19±133.89	0.001	7.166
	Control	932.94±201.28		

MDA-malondialdehyde; TAC-total antioxidant capacity; Std-standard deviation.

Table 3: Correlation analysis between the body mass index of the participants and their serum levels of MDA and TAC.

Correlation	r-value	p-value	Remark
MDA vs. BMI	0.189	0.138	Positive Correlation
TOTAL ANTIOXIDANT CAPACITY vs. BMI	0.108	0.399	Positive Correlation

**: Correlation is significant at the 0.01 level (2-tailed)



Abbreviations: MDA= malondialdehyde; TAC= total antioxidant capacity; PCOS= polycystic ovary syndrome. *=One participant in study group did not had final assessment for MDA and TAC.

Figure 1: Flow Chart of the participants*

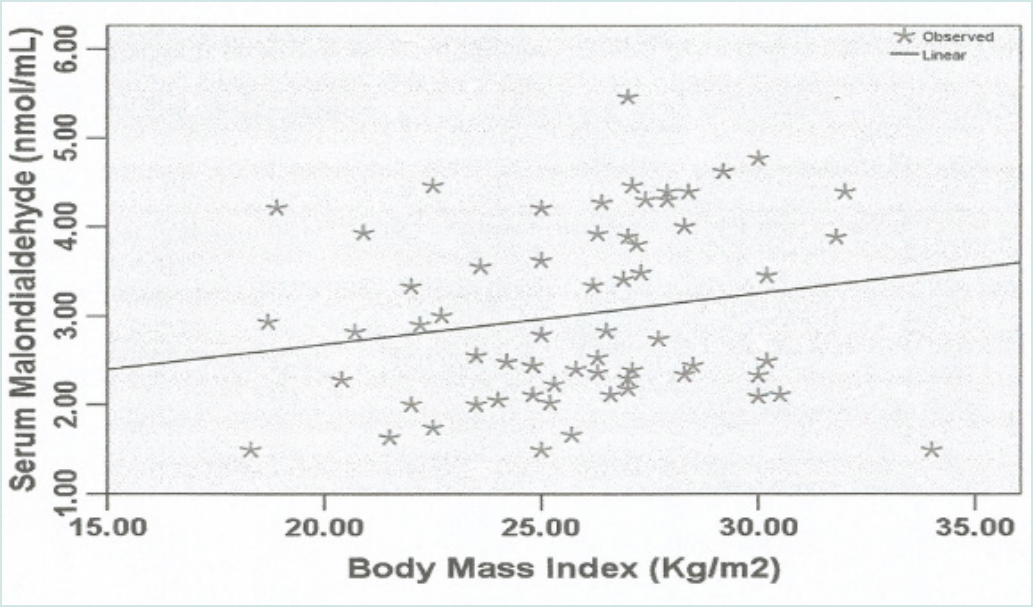


Figure 2: Scatter diagram shows the correlation between the serum malondialdehyde (nmol/mL) (Y-axis) and body mass index (kg/m2) (X-axis).

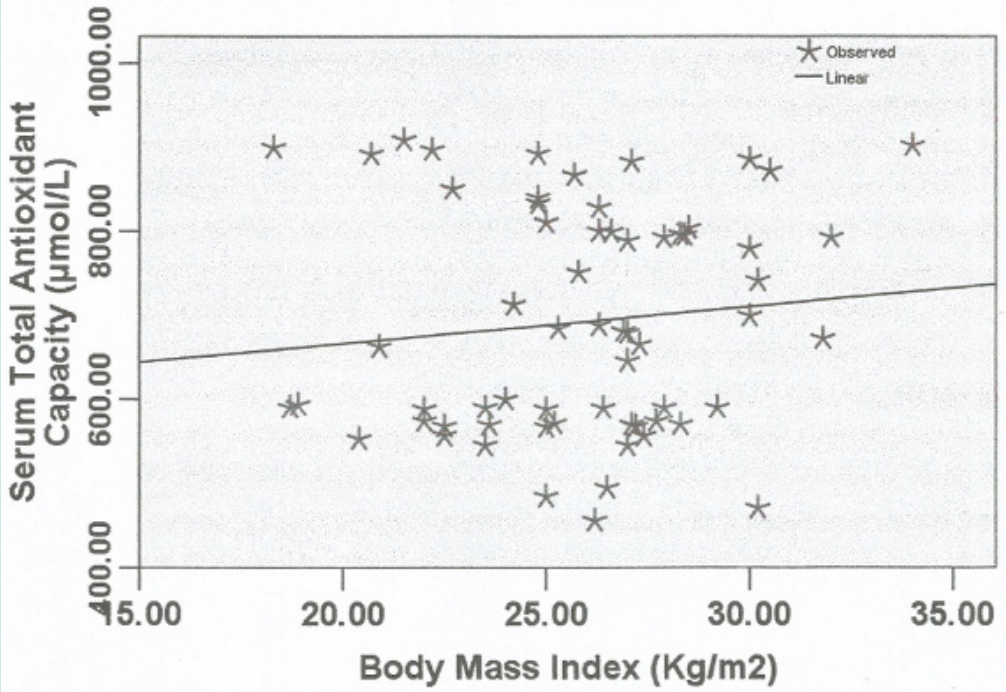


Figure 3: Scatter diagram revealing the correlation between the Serum Total Antioxidant Capacity (µmol/L) (Y-axis) and Body Mass Index (Kg/m2) (X-axis).

Discussion

The principal finding of the present study is that the mean serum levels of malondialdehyde in the PCOS infertility group and tubal factor infertility group was significantly different. Similarly, the mean level of total antioxidant capacity in PCOS infertility group versus tubal factor infertility also showed statistical significant difference. There was a weak positive correlation between serum malondialdehyde (nmol/mL) and body mass index) as well as serum total antioxidant capacity (µmol/L) and body mass index. This study shows that most of the women fall within the age bracket 26-30years with a mean of 28.84±5.76 years. This is akin to what was reported in previous Nigerian studies [4, 5,15]. Additionally, most the patients were nulliparous which is in line with an earlier Nigerian study by Igwegbe et al. [5] where 70% were nulliparous. It is not surprising because the study was amongst the infertility population. Thirty six out of the 63 participants in the test group were overweight while 6 out of the 63 participants were obese. This agrees with a study that showed that 73 of women were overweight [15]. Hence, improvement of lifestyle with dietary modification and exercise may invariably be helpful. In this study the total antioxidant capacity (TAC) status was significantly lower in the PCOS group (692.19±133.89µmol/L) than in the controls (932.94±201.25µmol/L). This finding is in line with that of Fankci et al. [16] but contrasted with the findings reported by Verit et al. [17] which indicated that TAC was higher in PCOS patients than in controls. This decrease in TAC may be explained by excessive production of oxidative stress markers, thereby overwhelming the antioxidant capacity of the body.

In this index study, the mean serum level of MDA was significantly increased in patients with PCOS compared to the control group (3.0+ 0.99 vs. 2.36+0.79 nmol/mL for test group and control group respectively; P-value 0.001). This is in keeping with the findings by Lai et al in a study that compared the MDA levels of patients with anovulatory (PCOS) infertility with that of tubal factor infertility among Chinese populations. This finding is also in line with a meta-analysis which revealed that the mean MDA levels per age and BMI were 47% increase in women with PCOS when compared with control [7]. Kuscu et al. [18] in their study which compared serum level of MDA between PCOS patients and healthy controls showed that MDA concentration was significantly higher in PCOS group. Similar findings were reported by several other studies by Sabuncu et al. [19], and Palacio et al. [20]. The authors suspected that the increased ROS expression level in the PCOS granulosa cells may have greatly induced cellular apoptosis [21] leading to a poor pregnancy outcome. On the other hand, Erdogan et al. [22] reported no significant difference in MDA and TAC level in patients with PCOS compared with the control group.

The plausible explanation of the no significant difference in the Erdogan et al report may be different gene polymorphism that varies from race to race. However, Turan et al [14] using similar Turkish population with PCOS and 30 healthy controls reported increase in MDA in patients with PCOS when compared with health controls. This study showed weak correlation between MDA and the BMI (MDA vs. BMI r=0.189; p-value 0.138) as well as TAC with the BMI (TAC vs. BMI=r=0.108; p-0.399). This contrasted with the findings of Priyank et al. [23] which showed positive correlation in TAC and MDA levels in obese patients with PCOS when compared

with normal control. The following limitations could be deduced from the study. It is possible that the difference in the concentration of oxidative stress markers may be a consequence rather than a cause of the disease [24]. This ambiguity makes it difficult to draw firm inferences on causality. Again, TBA (thiobarbituric acid) assay for the analysis of MDA has some drawbacks mainly, being a non-specific for MDA and not allowing distinction between free and bound components of MDA. Despite the above limitations, this study has several strengths. The findings of this study have highlighted the possible benefit of antioxidant therapy in the management of this common heterogeneous disorder that affects our women. Interventional study may be necessary to see if supplementation of antioxidants will be useful for this group of patients.

Conclusion

In conclusion, women with PCOS infertility have significantly higher serum levels of MDA and lower serum levels of TAC than women with tubal factor infertility. These findings support a possible role of oxidative stress markers in the etiology and/or pathogenesis of PCOS infertility. It also suggests a potential role of antioxidant supplementation in preventing the occurrence, halting the progression or in treating PCOS infertility. An interventional trial may be necessary to see if supplementation of antioxidants will be useful for this group of patients.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

Conceptualization: ROE, GUE, JOU. Data curation: CCO. Formal analysis: ROE, GUE, OSU. Methodology: OSU, JII, EOU. Project administration: CCO. Visualization: ROE, GUE, JOU, EOU, CGO, CIE, AAO, CIO, MEN, NLO, CME, COE, BUO, SON, MCE, JEM, IJO. Writing–original draft: ROE, GUE. Writing–review & editing: all authors.

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Consent for publication

All the participants gave consent for publication.

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Data Sharing Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Statement of Informed Consent

Written informed consent was obtained from all individual participants included in the study.

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